Ethanol Metabolism in Plant Tissues [1, 2]

E. A. Cossins [3] & Harry Beevers

Department of Biological Sciences, Purdue University, Lafayette, Indiana

Many plant tissues are known to produce ethanol when they are subjected to experimental stresses such as low oxygen supply or a variety of respiratory inhibitors (2). Ethanol production has also been observed under natural conditions in such tissues as germinating seeds, fruits, and root tips (10). The question whether ethanol, particularly that accumulated in periods of natural or imposed anaerobiosis, can be metabolized when the tissue subsequently gains better access to O_2 is still unanswered (10).

In earlier experiments, Cossins and Turner (5, 7) showed that in a variety of germinating seedlings, previously accumulated ethanol was consumed by the tissues, and by providing ethanol-2-C^{14} (6) to pea cotyledons they were able to show extensive conversion to a variety of products, including acetaldehyde, acids of the tricarboxylic acid cycle, and amino acids.

In this investigation, some twelve tissues, including storage organs, parts of seedlings, coleoptiles, fruits, roots, stems, and leaves have been examined for their ability to metabolize ethanol-C^{14} and the products of its utilization. Without exception, these materials converted part of the added ethanol to CO_2, organic acids, amino acids, and other products in periods of 4 hours or less. Distinctive differences in the patterns and rates of utilization were observed; in some tissues the rates of utilization were sufficiently high that the added ethanol (15 μmoles) was completely metabolized.

Materials & Methods

Plant Materials. Peas, (*Pisum sativum* L. var. Alaska) castor beans (*Ricinus communis* L. var. Cimmaron), and corn (*Zea mays* L. var. wf9 × 38-11 single cross hybrid) seeds were soaked overnight at 25° in tap water and then surface sterilized by washing in 0.1 % mercuric chloride solution w/v, followed by three successive washings in distilled water. The pea and corn seeds were sown in pots of garden soil and germinated at 25° in the greenhouse. For the experiments using 1 to 3-day old pea and corn seedlings, the seeds were germinated between layers of moist filter paper at 25° in the dark. The castor bean seeds were germinated in moist vermiculite at 25° in the dark for 5 days. The apples (*Pyrus malus* L.), potatoes (*Solanum tuberosum* L.) and carrots (*Daucus carota* L.) were obtained from commercial sources.

Feeding Experiments. In all experiments, the plant materials were sliced in order to facilitate the penetration of the ethanol-1-C^{14} solutions. Potato, carrot, and apple tissues were cut into cylinders, 6 mm in diameter and these were cut into 1 mm-thick slices using a razor blade. The cotyledons of peas and the endosperm of castor beans were also cut into 1 mm-thick slices. Coleoptiles and stem tissues were cut into 2 mm-thick sections and the pea and corn leaves were cut transversely into sections 2 mm wide. Corn root tips (5 mm) were excised from 3-day old seedlings grown in the dark. In all cases the prepared plant materials were washed twice in distilled water after sectioning and blotted dry with filter paper.

Ethanol-1-C^{14} was supplied by Volk Radio-Chemical Company and by Nichem, Inc. The ethanol-1-C^{14} solutions from the supplier were diluted with distilled water to give 150 μmoles of ethanol-1-C^{14} per milliliter of solution.

Slices (0.5 g fr wt) of the plant materials were incubated with 15 μmoles of ethanol-1-C^{14} (activities as indicated in table X). The incubations were carried out in large Warburg flasks (125 ml) at 25° in the dark. The only liquid in the flasks was 0.1 ml of ethanol-1-C^{14} solution, delivered from a micro syringe directly onto the plant material which just covered the bottoms of the flasks. Carbonate-free 20 % w/v NaOH solution was added to the center wells to absorb $C^{14}O_2$. The absorbed carbonate was converted to $BaCO_3$ and assayed for radioactivity on sintered porcelain planchets with the use of a Mylar window continuous gas flow Geiger-Muller tube. The counts were corrected for background and self absorption.

Analytical Methods. At the end of the experimental treatments, the tissues were killed by addition of 20 ml of boiling 80 % ethanol and ground finely in a hand blendor. After centrifugation the residue was washed successively with 10 ml of ether, 10 ml of 50 % ethanol, and finally with 10 ml of distilled water.

Aliquots of the combined supernatant fluids were placed on metal planchets to determine the amounts of radioactivity. This fraction is referred to as ethanol solubles in the tables. After drying and

[1] Received Oct. 29, 1962.

[2] Supported by National Science Foundation Grant G-16149.

[3] Present address: Department of Botany, University of Alberta, Canada.

counting, 1 ml of 4 N acetic acid was added to the planchets followed by drying and counting. In several of the tissues examined, acidification resulted in loss of radioactivity from this fraction. Such losses might have been due to the presence of carbonate material and volatile acids. The ethanol-soluble fraction was then taken to dryness under vacuum at 40°. The dried extract was then washed twice with 15 ml of anhydrous ether. The material removed in the ether washings constitutes the lipid material referred to in the tables.

The material insoluble in ether was then dissolved in 20 ml of distilled water at 30° and separated into four fractions using ion exchange resins (3).

The amino acids were separated from the organic acids and sugars by passing the water-soluble material through a 6 × 1 cm column of Dowex AG 50W-X8 (hydrogen form). The organic acids and sugars, passing through the column in water were separated from each other using a column of Dowex 1-X10 (formate form) according to the procedure of Canvin and Beevers (3).

The amino acids were eluted from the Dowex AG 50W-X8 column using 50 ml of 2 M HCl. Concentration of these eluates under vacuum at 40° resulted in hydrolysis of asparagine and glutamine to aspartic and glutamic acids. The acidic amino acids, mainly glutamic and aspartic acids were then separated from the neutral and basic amino acids by passing the amino acid fractions through a 6 × 1 cm column of Dowex 1-X10 in the acetate form (3).

After thorough drying at 100°, samples of the insoluble residues were combusted in a Stutz and Burris apparatus (14) using the wet combustion reagents of Van Slyke and Folch (17). The $BaCO_3$ so formed was assayed for radioactivity as above.

Results

Carrot Tissue. The metabolism of ethanol solutions by carrot tissues was reported by Wetzel (18) who claimed to have demonstrated a conversion of ethanol to acetaldehyde and to acetic acid. Lowe and James (11) were unable to confirm these observations using 5% ethanol solutions supplemented by trace amounts of ethanol-1-C^{14}. In order to investigate the possible metabolism of ethanol by carrot tissues, we fed micromolar amounts of ethanol-1-C^{14} which were not supplemented with nonradioactive ethanol.

The carrots were sliced as described by Lowe and James (11) and incubated with ethanol-1-C^{14} solutions as described previously. The results of such an experiment are shown in table I from which it is quite clear that the supplied ethanol was rapidly consumed; some was converted to alcohol-insoluble material and to CO_2. The greatest amounts of C^{14} in the alcohol-soluble fraction were present in the organic acids, amino acid, and lipids, and these were further fractionated to give the results shown in table II. These data show that the major fates of ethanol-1-C^{14} were conversion to acids of the tricarboxylic cycle, related amino acids, and to long-chain fatty acids.

Table I

Metabolism of Ethanol-1-C^{14} by Carrot Slices

Incubated at 25° for 4 hours.

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	850,500	
Organic acids	176,000	16
Amino acids	315,000	30
Lipids	56,700	5
Sugars	5,600	0.5
Volatile on acidification	297,000	29
Residue	117,000	11
CO_2	76,000	7
Total C^{14} incorporated	1,043,000	

Table II

Incorporation of C^{14} From Ethanol-1-C^{14} by Carrot Tissue Slices Organic, Amino Acid, & Lipid Fractions

Incubated at 25° for 4 hours. Organic acids were separated by method of Palmer (13) and identified by co-chromatography with authentic organic acids using *n*-propanol : ammonia 60 : 40 v/v; phenol : water 8 : 3 v/v; *n*-butanol : acetic acid : water 4 : 1 : 5 v/v/v. Glyoxylic acid was separated by method of Turner and Quartley (16). Amino acids were separated by ion exchange chromatography (9, 3). Lipids saponified by methods of Newcomb and Stumpf (12).

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Organic acids		
Malic	81,000	10
Citric	42,000	5
Glyoxylic	620	0.07
Glycollic acid fraction	8,600	1
Succinic	20,000	3
Unidentified peak	4,400	0.5
Lipid		
Non-saponifiable	3,100	0.4
Saponifiable material	53,500	6
Amino Acids		
Glutamic & glutamine	173,100	21
Aspartic & asparagine	38,000	4.5
Neutral & basic	25,100	3

Potato Tubers. Barker and el-Saifi (1) have shown that under anaerobic conditions, potato tubers accumulate large amounts of lactic acid and only relatively small amounts of ethanol. When the tissues were placed in air after the anaerobic period, the lactic acid content was rapidly depleted. These authors also reported a very small decrease in the ethanol content over this period. In this study the ability of potato tubers to utilize ethanol aerobically was examined (table III). Although the amount of C^{14} incorporated by the tuber slices was considerably lower than that observed in the experiment with carrot slices, the appearance of C^{14} in the various fractions shows that metabolism of the added ethanol-1-C^{14} had occurred. Only small amounts of radio-

Table III

Metabolism of Ethanol-1-C^{14} by Potato Tuber Slices
Incubated at 25° for 4 hours.

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	158,000	83
Organic acids	34,000	18
Acidic amino acids	51,000	27
Neutral & basic amino acids	8,400	4
Lipids	7,800	4
Sugars	1,600	0.8
Volatile on acidification	32,000	16
Residue	27,000	14
CO_2	4,400	2
Total C^{14} incorporated	189,000	

activity were present in the CO_2 released over the 4-hour experimental period but the acidic amino acid and organic acid fractions possessed considerable radioactivity.

Pea Cotyledons. Earlier experiments with ethanol-2-C^{14} (8) indicated a high rate of ethanol metabolism by pea cotyledons when high levels of endogenous ethanol were being depleted. In all the experiments, however, the amounts of C^{14} lost as $C^{14}O_2$ were negligible. In the experiments reported below, ethanol-1-C^{14} was employed to ascertain whether increased amounts of $C^{14}O_2$ would be involved and also whether ethanol fed to peas with a high endogenous ethanol content would be metabolized at a lower rate than by peas with a low endogenous ethanol content. The results are shown in table IV. The 1-day old cotyledons were soaked overnight in tap water, sliced, and incubated with ethanol-1-C^{14} solution. The 3-day old seedlings were soaked overnight in tap water and then placed on moist filter paper for 2 days at 25° to germinate. After such treatment the endogenous ethanol content was depleted (4).

In both the 1-day old cotyledons in air and the 3-day old cotyledons in O_2, there was an active incorporation of C^{14} from the ethanol-1-C^{14} solutions. In both cases, however, the percentage of C^{14} evolved as CO_2 was small. In the 1-day old cotyledons there was considerable incorporation into the organic acids fraction and into the insoluble residue. In the 3-day old cotyledons, there was a striking incorporation into the lipid fraction. The large amount of radioactivity in the lipids may so be related to the increase in fats, which occurs at this stage of germination in peas (4).

Castor Bean Endosperm. In preliminary experiments, Cossins and Turner (7) reported a loss of ethanol content as castor bean seedlings germinated. It would appear therefore that castor bean seedlings utilize ethanol during germination and this possibility was investigated further with ethanol-1-C^{14} (table V).

The 5-day old endosperm slices showed an active metabolism of the supplied ethanol-1-C^{14} under the conditions of the experiment. Considerable radioactivity was lost from the ethanol-soluble fraction on acidification with acetic acid followed by drying. Apart from this unidentified volatile material, the sugars had the highest radioactivity of the ethanol-soluble fraction. Only low C^{14} contents were present in the lipid, amino acid, and organic acid fractions. The similarity between the percentages for incorpora-

Table V

Metabolism of Ethanol-1-C^{14} by Castor Bean Endosperm Slices

Slices of endosperm from 5-day old castor beans were incubated in air at 25° for 4 hours.

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	165,000	64
Organic acids	7,000	3
Acidic amino acids	3,000	1
Neutral & basic amino acids	8,000	3
Lipids	6,000	2
Sugars	43,500	17
Volatile on acidification	70,000	27
Residue	57,000	22
CO_2	33,000	13
Total C^{14} incorporated	255,000	

Table IV

Metabolism of Ethanol-1-C^{14} by Pea Cotyledon Slices

Fraction	1-day old cotyledons*		3-day old cotyledons**	
	C^{14} (cpm)	% of incorporated C^{14}	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	145,000	69	377,000	93
Organic acids	72,000	34	76,100	18
Acidic amino acids	45,000	27	25,700	6
Neutral & basic amino acids	4,500	2	1,900	0.4
Lipids	6,100	3	268,000	66
Sugars	3,700	2	5,200	1
Residue	62,000	29	21,000	5
CO_2	2,400	1	5,000	1
Total C^{14} incorporated	209,000		403,000	

* Incubated in air at 25° for 4 hours.
** Incubated in O_2 at 25° for 1 hour.

tion into sugars and CO_2 would strongly suggest that ethanol was involved in the reactions of the pathway leading to sugar synthesis in these tissues as demonstrated by Canvin and Beevers (3).

Apple Fruits. When apple fruits were placed under anaerobic conditions and then into air for periods up to 14 days, Thomas (15), reported no loss of the accumulated ethanol and acetaldehyde. It was therefore of interest using the more sensitive methods of feeding micromolar amounts of ethanol-1-C^{14}, to determine whether apple fruits possessed any ability to metabolize ethanol. Slices of apple fruit tissue were incubated with ethanol-1-C^{14} as indicated in table VI.

Table VI

Metabolism of Ethanol-1-C^{14} by Apple Slices

Slices of apple tissue incubated at 25° in air for 4 hours.

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	8,500	95
Organic acids	340	4
Acidic amino acids	320	4
Neutral & basic amino acids	1,300	15
Lipids	380	4
Sugars	410	5
Volatile on acidification	5,000	58
Residue	Not active	
CO_2	420	5
Total C^{14} incorporated	8,900	

The slices of apple fruits showed only a low rate of ethanol metabolism under the conditions of the experiment. However, large amounts (58 %) of the total radioactivity incorporated, were lost when the ethanol-soluble extract was dried after addition of acetic acid. This loss can probably be attributed to volatile acids which contained the bulk of the C^{14} incorporated by the apple fruits.

Corn Coleoptile. The coleoptiles of 3-day old corn seedlings grown in the dark at 25° were excised and ethanol-1-C^{14} solutions added as described earlier. As shown in table VII, considerable metabolism of the added ethanol-1-C^{14} solution occurred. Only a small percentage of the total radioactivity was lost from the ethanol-soluble extract on adding acetic acid followed by drying. Large amounts of radioactivity were present in the organic acids, amino acid, and lipid fractions. The insoluble residual material contained a high percentage of the total C^{14} incorporated and in contrast to pea cotyledons (table IV) there was a considerable (35 %) release of the label $C^{14}O_2$.

Table VII

Metabolism of Ethanol-1-C^{14} by Corn Coleoptiles

Incubated in air at 25° for 4 hours.

Fraction	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	769,000	37
Organic acids	259,000	12
Acidic amino acids	115,000	6
Neutral & basic amino acids	123,000	6
Lipids	156,000	8
Sugars	15,000	0.7
Volatile on acidification	46,000	2
Residue	551,000	27
CO_2	733,000	35
Total C^{14} incorporated	2,053,000	

Pea Seedlings. The incorporation of C^{14} from ethanol-1-C^{14} by pea leaves and shoots is presented in table VIII.

It is evident that the shoots and leaves of 4-day old pea seedlings actively utilized the ethanol-1-C^{14} added. In both tissues there was an active conversion to CO_2. The distinguishing features of the ethanol metabolism in these tissues was the considerable incorporation into the insoluble residue by the stem tissues and into the lipids by the leaves.

Corn Seedlings. It is evident from table IX that corn root tips, shoots, and leaves all showed a striking utilization of the supplied ethanol-1-C^{14} in the 4-hour experimental period. The greatest incorporation of the C^{14} was shown by the shoots with lesser amounts in the leaves and root tips. In the shoots considerable incorporation (61 % of the total C^{14}

Table VIII

Metabolism of Ethanol-1-C^{14} by 14-Day Old Pea Seedlings

Sections of shoots and leaves incubated at 25° in air for 4 hours.

Fraction	Shoots		Leaves	
	C^{14} (cpm)	% of incorporated C^{14}	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	450,000	57	308,000	75
Organic acids	89,000	11	26,000	6
Acidic amino acids	80,000	10	26,000	6
Neutral & basic amino acids	26,000	3	8,400	2
Lipids	100,000	13	151,000	37
Sugars	57,000	7	42,000	10
Volatile on acidification	56,000	7	42,000	10
Residue	214,000	27	70,000	17
CO_2	125,000	16	33,000	8
Total C^{14} incorporated	800,000		411,000	

Table IX

Metabolism of Ethanol-1-C^{14} by Corn Seedlings

Five-day old corn seedlings dissected into root tips, shoots, and leaves, incubated in air at 25° for 4 hours.

Fraction	Root tips		Shoots		Leaves	
	C^{14} (cpm)	% of incorporated C^{14}	C^{14} (cpm)	% of incorporated C^{14}	C^{14} (cpm)	% of incorporated C^{14}
Ethanol solubles	178,500	75	190,000	30	206,000	48
Organic acids	36,100	15	28,000	4	40,500	9
Acidic amino acids	28,000	12	18,000	3	62,500	14
Neutral & basic amino acids	23,000	10	7,700	1	16,000	4
Lipids	10,600	4	55,500	9	48,000	11
Sugars	8,000	3	10,700	2	21,000	5
Volatile on acidification	67,000	28	37,000	6	...	...
Residue	39,000	16	381,000	61	130,000	32
CO_2	22,100	9	57,500	9	96,000	22
Total C^{14} incorporated	240,000		629,000		432,000	

utilized) occurred into the insoluble residue, whereas this fraction accounted for 32 % in the leaves and 16 % in the root tips. In the ethanol-soluble extract prepared from the corn leaves, no activity was lost when acetic acid was added followed by drying. Corn seedlings therefore showed marked utilization of ethanol by the root tips, shoots, and leaves and also by the coleoptiles (table VII).

Discussion

The results clearly show that all the tissues examined possessed the ability to metabolize the micromolar amounts of ethanol-1-C^{14} supplied. In some tissues nearly all the ethanol-1-C^{14} supplied during the experimental periods was utilized (table X). In the corn coleoptiles and pea shoots, 93 % of the radioactivity supplied was recovered from the fractions separated after 4 hours. Contrary to an earlier report (11) the carrot tissues utilized 86 % of the ethanol-1-C^{14} during the 4 hours incubation in O_2. Other high rates of ethanol metabolism were shown by the corn shoots, corn leaves, castor bean endosperm, potato slices, and the pea cotyledons. The earlier reports of a possible utilization of ethanol by castor bean seeds (7) and by potato tubers (1) are therefore confirmed. The very small amounts of ethanol metabolized by the apple fruit slices are in agreement with the earlier work of Thomas (15) who was unable to demonstrate a loss of ethanol from apple fruits placed in air after periods of anaerobiosis.

With the exception of the corn leaves and coleoptiles, the amounts of $C^{14}O_2$ evolved in the experiments were small when compared with the active incorporation of C^{14} into the other fractions separated. It is of interest that the percentages of isotope evolved as $C^{14}O_2$ by these tissues were small, especially as the

Table X

Percentage of Ethanol-1-C^{14} Utilized by Higher Plant Tissues

Tissue	cpm of ethanol-1-C^{14} added	cpm utilized	% of ethanol-1-C^{14} utilized
Storage tissues			
Carrot**	1,200,000	1,043,000	86
Potato	450,000	189,000	42
Germinating seedlings			
1-day old pea cotyledon	850,000	209,000	24
3-day old pea cotyledon*	675,000	403,000	60
Castor bean endosperm	450,000	255,000	57
Fruit tissues			
Apple	450,000	8,900	2
Coleoptile tissues			
Corn	2,200,000	2,053,000	93
Shoots, roots & leaves			
Pea shoots	850,000	800,000	93
Pea leaves	850,000	411,000	49
Corn root tips	850,000	240,000	28
Corn shoots	850,000	629,000	74
Corn leaves	850,000	432,000	51

* Slices incubated at 25° in O_2 for 1 hour.
** Incubated at 25° in O_2 for 4 hours.
All other treatments incubated at 25° in air for 4 hours.

ethanol was labeled in the carbinol group. Possible differences in the incorporation of C^{14} from ethanol-1-C^{14} and acetate-1-C^{14} by these tissues will be investigated in the future.

Ethanol can therefore be no longer regarded as an end product of metabolism. The connection between possible ethanol metabolism and studies with auxin dissolved in ethanol has been emphasized in another paper (8). Clearly all the tissues investigated were able to convert the supplied ethanol-1-C^{14} into organic acids, amino acids, lipids, and sugars. These observations are consistent with the conversion of ethanol to acetyl coenzyme A which is then involved in the reactions of established pathways.

Summary

I. Ethanol-1-C^{14} was rapidly metabolized by carrot disks, pea cotyledons, castor bean endosperm, corn coleoptiles, pea shoots, potato tubers, and corn shoots. A low rate of ethanol-1-C^{14} metabolism was detected in apple tissue.

II. In all tissues, the ethanol-soluble fractions contained a large percentage of the C^{14} derived from ethanol-1-C^{14}. With the exception of corn coleoptile, only small amounts of radioactivity were present in the CO_2 evolved.

III. In pea cotyledon ethanol-1-C^{14} was converted predominately into fat. In castor bean endosperm there was a striking incorporation into sugars. In corn shoots the major repository of the label was the insoluble residual material.

IV. The results are interpreted as being consistent with conversion of ethanol-1-C^{14} to acetyl coenzyme A, which is then metabolized by established pathways.

Literature Cited

1. Barker, J. & A. F. el-Saifi. 1953. Studies in the respiratory & carbohydrate metabolism of plant tissues. III. Experimental studies of the formation of CO_2 & of changes in lactic acid & other products in potato tubers in air following anaerobic conditions. Proc. Roy. Soc. (London), Ser. B. 140: 508–22.
2. Betz, A. 1960. Encyclopedia of plant physiology. Springer-Verlag Berlin. vol. XII/2: 88–113.
3. Canvin, D. T. & H. Beevers. 1961. Sucrose synthesis from acetate in the germinating Castor Bean. Kinetics & Pathway. J. Biol. Chem. 236: 988–95.
4. Cossins, E. A. 1961. Metabolism of ethanol by higher plant tissues. Doctoral Thesis. University of London.
5. Cossins, E. A. & E. R. Turner. 1959. Utilization of alcohol in germinating pea seedlings. Nature 183: 1599–600.
6. Cossins, E. A. 1962. Utilization of ethanol-2-C^{14} by pea slices. Nature 194: 1095–96.
7. Cossins, E. A. & E. R. Turner. 1962. Losses of alcohol & alcohol dehydrogenase activity in germinating seeds. Ann. Botany (in press).
8. Cossins, E. A. & E. R. Turner. 1962. The metabolism of ethanol in germinating pea seedlings. J. Exptl. Botany (in press).
9. Hirs, C. H. W., S. Moore, & W. H. Stein. 1954. The chromatography of amino acids on ion exchange resins. Use of volatile acids for elution. J. Am. Chem. Soc. 76: 6063–65.
10. James, W. O. 1953. Plant Respiration. Oxford University Press.
11. Lowe, C. & W. O. James. 1960. Carrot tissue & ethanol. New Phytologist 59: 288–93.
12. Newcomb, E. H. & P. K. Stumpf. 1953. Fat metabolism in higher plants. I. Biogenesis of higher fatty acids by slices of peanut cotyledons in vitro. J. Biol. Chem. 200: 233–39.
13. Palmer, J. K. 1955. Chemical investigations of the Tobacco plant. X. Determination of organic acids by ion exchange chromatography. Conn. Agr. Expt. Sta. Bull. 589.
14. Stutz, R. E. & R. H. Burris. 1951. Photosynthesis & metabolism of organic acids in higher plants. Plant Physiol. 26: 226–43.
15. Thomas, M. 1925. The controlling influence of carbon dioxide. V. A quantitative study of the production of ethyl alcohol & acetaledhyde by cells of higher plants. Biochem. J. 19: 927–47.
16. Turner, E. R. & C. E. Quartley. 1956. An inhibition of respiration in peas induced by oxygen poisoning. J. Exptl. Botany 7: 362–71.
17. Van Slyke, D. D. & J. Folch. 1940. Manometric carbon determination. J. Biol. Chem. 136: 509–41.
18. Wetzel, K. 1933. Zur Physiologie der anaeroben Atmung Höherer Pflanzen. Ber. Deut. Botan. Ges. 51: 46–51.